

What is claimed is:

1. A non-naturally occurring composition of matter comprising at least one component possessing nucleic acid polymerase enhancing activity selected from the group consisting of: an isolated or purified naturally-occurring polymerase enhancing protein obtained from a bacterial, eukaryotic, or archael source; a wholly or partially synthetic protein having the same amino acid sequence as said naturally-occurring protein or an analog thereof possessing polymerase enhancing activity; polymerase-enhancing mixtures of one or more of said naturally occurring or wholly or partially synthetic proteins; a polymerase-enhancing protein complex of one or more of said naturally occurring or wholly or partially synthetic proteins; and a polymerase enhancing partially purified cell extract containing one or more of said naturally occurring proteins.
2. A composition of matter according to claim 1, wherein said component possessing polymerase enhancing activity is a cell extract.
3. A composition of matter according to claim 2, wherein said cell extract is from an archael source.
4. A composition of matter according to claim 3, wherein said cell extract is from *Pyrococcus furiosus*.
5. A composition of matter according to claim 1, wherein said component possessing polymerase enhancing activity is a protein complex.
6. A composition of matter according to claim 5, wherein said protein complex is from an archael source.
7. A composition of matter according to claim 6, wherein said protein complex is from *Pyrococcus furiosus*.
8. A composition of matter according to claim 7, wherein said protein complex is P300.
9. A composition of matter according to claim 5, wherein said protein complex comprises a plurality of subunits wherein at least one subunit has a molecular weight of approximately 17-18kD in the fully denatured, monomeric form.
10. A composition of matter according to claim 9, wherein a subunit has a sequence of amino acids at the amino terminal end comprising SEQ ID NO: 46.
11. A composition of matter according to claim 9, wherein a subunit has a sequence of amino acids comprising one of SEQ ID NO: 47 or 48.

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12. A composition of matter according to claim 10 or 11, further comprising a subunit encoded by a DNA comprising the nucleotide sequence of SEQ ID NO: 18, a degenerate variant thereof, or a sequence hybridizable to the complement of SEQ ID NO: 18.

13. A composition of matter according to claim 1, wherein said component possessing polymerase enhancing activity is a protein.

14. A composition of matter according to claim 13, wherein said protein is from an archael source.

15. A composition of matter according to claim 14, wherein said protein is from *Pyrococcus furiosus*.

16. A composition of matter according to claim 15, wherein said protein comprises at least one protein having a molecular weight of approximately 17-18kD in the fully denatured, monomeric form.

17. A composition of matter according to claim 16, wherein said protein is selected from the group consisting of: a protein having a sequence of amino acids at the amino terminal end or within about 20 amino acids from the amino terminal end comprising one of SEQ ID NO: 69 or 11; a protein encoded by a nucleic acid comprising the sequence of SEQ ID NO: 70, a degenerate variant thereof, or a sequence hybridizable to the complement of the sequence of SEQ ID NO: 70; or a protein having a sequence of amino acids comprising SEQ ID NO: 71.

18. A composition of matter according to claim 1, wherein said component possessing polymerase enhancing activity is a wholly or partially synthetic protein comprising the same amino acid sequence as said naturally-occurring protein or an analog thereof.

19. A composition of matter according to claim 18, wherein said protein has a molecular weight of approximately 17-18kD in the fully denatured, monomeric form.

20. A composition of matter according to claim 19, wherein said protein comprises a sequence of amino acids at the amino terminal end or within 20 amino acids of the amino terminal end comprising one of SEQ ID NO: 11 or 69.

21. A composition of matter according to claim 19, wherein said protein has a sequence of amino acids comprising SEQ ID NO: 71.

22. A composition of matter according to claim 20 or 21, further comprising a subunit encoded by a DNA comprising the nucleotide sequence of SEQ ID NO: 70.

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23. A composition of matter according to claim 1, wherein said component possessing polymerase enhancing activity is a mixture of proteins.

24. An isolated or purified DNA comprising a sequence encoding a protein according to the protein of one of claims 16 or 17.

✓25. An isolated or purified DNA comprising a sequence selected from the group consisting of: the sequence set forth in SEQ ID NO: 18, a degenerate sequence thereof, or a DNA sequence hybridizable to the complement of SEQ ID NO: 18; the sequence set forth in SEQ ID NO: 70, a degenerate sequence thereof, or a DNA sequences hybridizable to the complement of SEQ ID NO: 70.

26. An isolated or purified DNA sequence hybridizable to the complement of a DNA sequence according to claim 24 under stringent conditions.

27. An isolated or purified DNA sequence hybridizable to the complement of a DNA sequence encoding a protein according to claim 20.

28. A composition of matter comprising a polymerase-enhancing protein encoded by DNA according to claim 26.

29. A composition of matter comprising a polymerase-enhancing protein encoded by DNA according to claim 27.

30. A non-naturally occurring composition comprising a polymerase-enhancing composition according to claim 1 and one or more DNA polymerases.

31. A composition according to claim 30, wherein at least one of said polymerases is a thermostable DNA polymerase.

32. A composition according to claim 30, wherein at least one of said polymerases is derived from an archael source.

33. A composition according to claim 31, wherein at least one of said polymerases is a DNA polymerase derived from the *Pyrococcus* species or the *Thermococcus* species.

34. A mixture according to claim 31, wherein at least one of said polymerases is *Pyrococcus furiosus*, *Pyrococcus* sp. JDF3, *Pyrococcus* sp. GBD, *Pyrococcus* sp. KOD, *Thermococcus litoralis*, or *Pyrococcus woesei* DNA polymerase.

✓35. A kit for replicating nucleic acids comprising a polymerase-enhancing composition of claim 1 and at least one nucleic acid polymerase.

36. A kit according to claim 35, comprising at least one recombinant nucleic acid polymerase.

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37. A kit according to claim 35 or 36, which can be used in a site-directed mutagenesis method.
38. A kit according to claim 35 or 36, which can be used in a nucleic acid sequencing method.
39. A kit according to claim 35 or 36, which can be used in an amplification reaction.
40. A method of enhancing a nucleic acid polymerase reaction comprising, in any appropriate order, combining (a) a nucleic acid sequence template for a nucleic acid polymerase, (b) at least one nucleic acid polymerase, and (c) a polymerase enhancing composition according to claim 1.
41. A method according to claim 40, wherein said reaction is a replication reaction.
42. A method according to claim 40, wherein said reaction comprises an amplification reaction.
43. A method according to claim 40, wherein said reaction comprises a PCR process or RT-PCR process.
44. A method according to claim 41, 42, or 43, further comprising a site-directed mutagenesis process, a cycle sequencing process, or a cloning process.
45. An antibody that binds to a composition of matter of claim 1.
46. An antibody that binds to a protein having an amino acid sequence comprising one of SEQ ID NO: 19 or 71.
47. A method for purifying a polymerase-enhancing protein comprising:
 - (a) solubilizing the protein from archae cells while substantially maintaining protein:protein interactions;
 - (b) performing heparin sepharose chromatography on the product of (a);
 - (c) performing size exclusion chromatography on the product of (b); and
 - (d) identifying a polymerase enhancing activity.
48. A method for identifying the presence or absence of a composition of matter with polymerase enhancing activity, comprising adding a protein extract from cells to a nucleic acid polymerization reaction and measuring the number of products produced in said polymerization reaction compared to the number of products in a control reaction.

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49. The method of claim 48, wherein the polymerase activity employed in said nucleic acid polymerization reaction comprises activity of at least one of native or cloned bacterial DNA polymerase, native or cloned archaeal DNA polymerase, native or cloned polymerase from the *Pyrococcus* species or the *Thermococcus* species, *Pyrococcus furiosus* DNA polymerase, native or cloned reverse transcriptase, or native or cloned RNA polymerase.

50. The method of claim 48, wherein the polymerization reaction comprises one of a PCR process or RT-PCR process.

51. The method of claim 49, wherein the polymerization reaction comprises one of a PCR process or RT-PCR process.

✓52. A method for identifying DNA encoding polymerase-enhancing activity in a sample comprising contacting a nucleic acid probe comprising all or a portion of the sequence of nucleotides from SEQ ID NO: 70 or a complement thereof, or comprising a sequence of nucleotides encoding all or a portion of the amino acid sequence of SEQ ID NO: 71 or a complement thereof, to nucleic acids of the sample and isolating a nucleic acid capable of hybridizing to said probe.

53. A method as claimed in claim 52, wherein a hybridization condition is employed comprising a low stringency wash in a solution comprising approximately .45 M NaCl, approximately .045 M trisodium citrate, and approximately .1% SDS, and wherein the wash temperature is approximately 37° to approximately 42°C.

54. A method as claimed in claim 53, wherein a hybridization buffer is employed comprising approximately .75M NaCl, approximately .075 M trisodium citrate, and approximately 50% formamide, and wherein a hybridization wash is employed comprising approximately .1 M phosphate, approximately .1 x SET, approximately .1% sodium pyrophosphate, and approximately .1% SDS at approximately 45°C.

55. A method for identifying DNA encoding material having polymerase-enhancing activity in a sample comprising performing an amplification reaction with at least one primer capable of hybridizing to a DNA encoding material having polymerase-enhancing activity or a protein component thereof.

56. A method as claimed in claim 55, wherein at least one primer comprises an at least 15 nucleotide portion of the DNA of SEQ ID NO: 70, or a DNA comprising a sequence encoding an at least 5 amino acid portion of SEQ ID NO: 71, or a DNA comprising the DNA of one of claims 25-27.

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57. A method for identifying polymerase-enhancing activity in a sample comprising contacting an antibody of claim 45 or 46 with said sample and detecting protein binding to said antibody.

58. A DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector.

59. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the protein is in monomeric, dimeric, or multimeric form.

60. A P45 protein as claimed in claim 59, wherein the cell is a bacterial cell.

61. A PEF complex comprising a P45 protein as claimed in claim 59.

62. An antibody that binds to a P45 protein as claimed in claim 59.

63. An antibody that binds to a PEF complex as claimed in claim 61.

64. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the P45 protein is produced as a fusion protein.

65. A P45 protein as claimed in claim 64, wherein the fusion protein comprises a calmodulin binding peptide.

66. A P45 protein as claimed in claim 65, wherein the expression vector is pCAL-n-EK.

67. A kit for replicating nucleic acids comprising at least one polymerase, a P45 protein as claimed in claim 59, and reagents for performing a polymerization reaction.

68. A kit as claimed in claim 67, wherein the P45 protein is present in a PEF complex.

69. A method of enhancing a nucleic acid polymerase reaction comprising adding a P45 protein as claimed in claim 59 to a polymerization reaction.

70. A method of enhancing a nucleic acid polymerase reaction as claimed in claim 69, wherein the P45 protein is present in a PEF complex.

71. A method of enhancing a nucleic acid polymerase reaction comprising performing the reaction in the presence of one or more of the following: a PEF; dUTPase activity; a protein that turns-over dUTP; a protein having one or more of SEQ ID NO: 72-81.

72. A method as claimed as claimed in claim 71, wherein the dUTPase activity comprises one or more of a P45 protein produced from a cell containing a DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector, wherein the protein is in monomeric, dimeric, or multimeric form, a human dUTPase, a

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57. A method for identifying polymerase-enhancing activity in a sample comprising contacting an antibody of claim 45 or 46 with said sample and detecting protein binding to said antibody.

1R
15x
C-terminal
58. A DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector.

59. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the protein is in monomeric, dimeric, or multimeric form.

60. A P45 protein as claimed in claim 59, wherein the cell is a bacterial cell.

61. A PEF complex comprising a P45 protein as claimed in claim 59.

62. An antibody that binds to a P45 protein as claimed in claim 59.

63. An antibody that binds to a PEF complex as claimed in claim 61.

64. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the P45 protein is produced as a fusion protein.

65. A P45 protein as claimed in claim 64, wherein the fusion protein comprises a calmodulin binding peptide.

66. A P45 protein as claimed in claim 65, wherein the expression vector is pCAL-n-EK.

67. A kit for replicating nucleic acids comprising at least one polymerase, a P45 protein as claimed in claim 59, and reagents for performing a polymerization reaction.

68. A kit as claimed in claim 67, wherein the P45 protein is present in a PEF complex.

69. A method of enhancing a nucleic acid polymerase reaction comprising adding a P45 protein as claimed in claim 59 to a polymerization reaction.

70. A method of enhancing a nucleic acid polymerase reaction as claimed in claim 69, wherein the P45 protein is present in a PEF complex.

71✓ A method of enhancing a nucleic acid polymerase reaction comprising performing the reaction in the presence of one or more of the following: a PEF; dUTPase activity; a protein that turns-over dUTP; a protein having one or more of SEQ ID NO: 72-81.

72. A method as claimed as claimed in claim 71, wherein the dUTPase activity comprises one or more of a P45 protein produced from a cell containing a DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector, wherein the protein is in monomeric, dimeric, or multimeric form, a human dUTPase, a

Spec out
need
Seq
ID No.
Specifically
Identify
P45
Nucleic
acid

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bacterial dUTPase, an archaeal dUTPase, a yeast dUTPase, a mammalian dUTPase, or an animal dUTPase.

73. A method for controlling the activity of a polymerase in a polymerization reaction, comprising changing the amount of dUTP present or generated during the reaction by adding a material having PEF activity.

74. A method as claimed in claim 73, wherein the PEF activity comprises a P45 protein produced from a cell containing a DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector, wherein the protein is in monomeric, dimeric, or multimeric form.

75. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP or dCTP and monitoring the inorganic pyrophosphate levels.

76. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP and monitoring any change in polymerization levels.

77. A non-naturally occurring composition of matter comprising a P45 protein.

78. A composition of matter as claimed in claim 77, wherein the P45 protein is in monomeric, dimeric, or multimeric form.

79. A composition of matter as claimed in claim 77, wherein the P45 protein is present in a protein complex.

80. A composition of matter as claimed in claim 77, wherein the P45 protein is an analog P45 protein.

81. A method of producing a P45 protein comprising transferring a DNA construct as claimed in claim 58 into a host cell and expressing the P45 protein.

82. A method as claimed in claim 81, wherein the P45 protein is expressed as a fusion protein.

83. A method of producing a PEF analog protein comprising introducing at least one mutation into the sequence encoding P45 protein of the DNA construct of claim 58 or into a sequence encoding a dUTPase protein, transferring the sequence to a host cell, and expressing the PEF analog protein.

84. A DNA encoding a PEF activity comprising (a) one or more of SEQ ID NO.: 32-35, 82, 83, or 70, (b) a sequence hybridizable to the complement of one or more those sequences under stringent conditions, or (c) a degenerate variant of (a) or (b).

art w/ 10 mos

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bacterial dUTPase, an archael dUTPase, a yeast dUTPase, a mammalian dUTPase, or an animal dUTPase.

73. A method for controlling the activity of a polymerase in a polymerization reaction, comprising changing the amount of dUTP present or generated during the reaction by adding a material having PEF activity.

74. A method as claimed in claim 73, wherein the PEF activity comprises a P45 protein produced from a cell containing a DNA construct comprising a sequence encoding PEF protein P45 operably linked to an expression vector, wherein the protein is in monomeric, dimeric, or multimeric form.

75. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP or dCTP and monitoring the inorganic pyrophosphate levels.

76. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP and monitoring any change in polymerization levels.

77. A non-naturally occurring composition of matter comprising a P45 protein.

78. A composition of matter as claimed in claim 77, wherein the P45 protein is in monomeric, dimeric, or multimeric form.

79. A composition of matter as claimed in claim 77, wherein the P45 protein is present in a protein complex.

80. A composition of matter as claimed in claim 77, wherein the P45 protein is an analog P45 protein.

81. A method of producing a P45 protein comprising transferring a DNA construct as claimed in claim 58 into a host cell and expressing the P45 protein.

82. A method as claimed in claim 81, wherein the P45 protein is expressed as a fusion protein.

83. A method of producing a PEF analog protein comprising introducing at least one mutation into the sequence encoding P45 protein of the DNA construct of claim 58 or into a sequence encoding a dUTPase protein, transferring the sequence to a host cell, and expressing the PEF analog protein.

84. A DNA encoding a PEF activity comprising (a) one or more of SEQ ID NO.: 32-35, 82, 83, or 70, (b) a sequence hybridizable to the complement of one or more those sequences under stringent conditions, or (c) a degenerate variant of (a) or (b).

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85. A protein having PEF activity comprising one or more of SEQ ID NO.: 72-81.

86. A method for cloning a PEF activity comprising employing the complement of one or more nucleic acids comprising one or more of SEQ ID NO: 32-35, 82, 83, or the complement of all or a portion of SEQ ID NO: 70, and identifying a clone containing a sequence that hybridizes to the one or more nucleic acids.

87. A PCR enhancing, protein extract comprising purified proteins from *Thermus thermophilis* that possesses dUTPase activity.

88. A protein extract of claim 87, which comprises a protein that can be bound by an antibody specific for recombinant Pfu P45 protein.

89. A protein extract of claim 88, wherein the protein possesses a molecular weight of approximately 24kD in an SDS-PAGE gel.

90. A protein extract of claim 87, which comprises a protein that possesses a molecular weight of approximately 24kD in an SDS-PAGE gel.

91. A composition comprising a protein extract as claimed in claim 87.

92. A composition comprising a protein extract as claimed in claim 87, and further comprising a thermostable DNA polymerase.

93. A composition comprising a protein extract of claim 88 and a thermostable DNA polymerase.

94. A computer readable medium having stored in it the full or partial amino acid or DNA sequence information of a PEF protein.

95. A computer-based method of screening for a PEF, comprising providing a computer readable medium as claimed in claim 94, and identifying other sequences in a database that possess sequence homology, similarity, or identity to all or a portion of the sequence stored in the computer readable medium.